BIOLOGICAL REMOVAL OF HYDROGEN SULFIDE FROM COAL SYNTHESIS GAS

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INTRODUCTION

Coal synthesis gas may typically contain up to 1% hydrogen sulfide (H_2S) which, in conventional processing, must be removed prior to methanation to prevent catalyst fouling (1,2). Alternatively, Barik et al (3) have shown that coal synthesis gas may be upgraded microbially in a process which is not particularly sensitive to H_2S. However, H_2S would have to be removed from the resulting upgraded gas prior to distribution and sale.

The work described here was initiated to investigate the feasibility of a microbial process for the removal and oxidation of H_2S from a gas stream such as the product of the shift conversion of coal gas or the product of the microbial methanation process. The high reaction rates and mild reaction conditions characteristic of a microbial process can potentially yield technical and economic advantages over conventional processes. Conceptually, a microbial process can replace an entire conventional gas processing train of H_2S removal (amine system), H_2S disposal (Claus or Stretford system) and tail gas clean-up or any individual processing step.

There are many bacteria capable of H_2S oxidation and therefore serve as potential candidates to form the basis of a microbial gas desulfurization technology. However, the ideal microorganism for this application must possess several other characteristics in addition to the ability to oxidize hydrogen sulfide if the technology is to be economically viable. The bacteria chosen for this study was Thiobacillus denitrificans.

T. denitrificans is a strict autotroph and facultative anaerobe first described by Baalsrud and Baalsrud (4). Thiosulfate, elemental sulfur and soluble sulfide may be utilized as energy sources with oxidation to sulfate which accumulates external to the cells. Under anaerobic conditions nitrate may be used as a terminal electron acceptor with reduction to elemental nitrogen. Reported here is a study of the stoichiometry and kinetics of aerobic and anaerobic oxidation of $H_2S(g)$ by T. denitrificans in batch and continuous flow reactors under sulfide-limiting conditions. The maximum loading of the biomass for aerobic and anaerobic oxidation of H_2S was determined and a study of reactor upset conducted. The effect of heterotrophic contamination was also examined. Lastly, T. denitrificans has also been immobilized by co-culture with floc-forming heterotrophs to produce macroscopic floc with excellent settling properties. A study of the oxidation of H_2S by immobilized T. denitrificans is also reported.

MATERIALS AND METHODS

Organism and Stock Cultures

Stock cultures of wild-type (ATCC 23642) and sulfide-tolerant strains of Thiobacillus denitrificans were grown anaerobically in thiosulfate maintenance medium in 10 ml culture tubes at 30°C. Thiosulfate medium is described in Table 1. The trace metal solution has been described elsewhere (5). Stocks were transferred every 30 days and stored at 4°C until used.

Oxidation of H_2S by Planktonic or Free-Cell T. denitrificans

All investigations of anaerobic growth of planktonic T. denitrificans on H_2S(g) as the energy source were conducted with a B. Braun Biostat M bench scale fermenter (culture volume, 1.4 l). Aerobic experiments also made use of an L. E. Marubishi MD 300 fermenter (culture volume, 2.0 l). In a typical anaerobic batch experiment, T. denitrificans was grown in thiosulfate maintenance medium at 30°C and pH 7.0 to an OD_{460} of 0.5-0.8 prior to the introduction of H_2S. This optical density (OD) corresponds to greater than 10^8-10^9 cells/ml. The purpose of this prior cultivation on thiosulfate was to develop a sufficient concentration of biomass in the reactor so that an appreciable rate of H_2S(g) could be fed to the reactor without exceeding the biooxidation capabilities of the biomass. Otherwise toxic levels of sulfide would accumulate in the culture. The OD level of 0.5-0.8 was initially chosen arbitrarily. While growing on thiosulfate a gas feed of 5 mol% CO_2 in nitrogen was normally fed to the reactor at 30 ml/min to ensure continuous availability of a carbon source. A hydrogen sulfide feed gas typically contained 0.5-1.0 mol% H_2S, 5 mol% CO_2, and balance N_2.

The pathways for sulfide and thiosulfate oxidation to sulfate in T. denitrificans are not independent but have two common intermediates, a membrane bound polysulfide and sulfite (6). In the presence of thiosulfate, the rate of sulfide oxidation would be reduced because of competition between intermediates of thiosulfate and sulfide oxidation for the same enzymes of the sulfur pathway. Therefore, prior to the introduction of H_2S to T. denitrificans cultures, residual thiosulfate was removed. This was accomplished by sedimenting the cells by centrifugation, washing with 20 mM phosphate buffer (pH 7.0) and resuspending the cells in the fermenter in thiosulfate maintenance medium without thiosulfate. The cell suspension was then purged for 1 h with 5 mol% CO_2 in nitrogen to allow residual thiosulfate to be metabolized before the feed gas was changed to include hydrogen sulfide. H_2S oxidizing cultures were also maintained at 30°C and pH 7.0.

The start-up of a continuous-flow, anaerobic reactor began with the establishment of a batch culture as described above. When stable operation on H_2S(g) feed was indicated, the reactor was switched to a continuous flow mode by introducing a continuous stream of nutrient solution (thiosulfate maintenance medium without thiosulfate) and withdrawing a continuous stream of reactor mixed liquor (cells plus medium) at the same rate. A schematic diagram

of the continuous flow reactor system used in these experiments is given in Figure 1.

In batch, aerobic experiments, T. denitrificans was grown in thiosulfate maintenance medium without nitrate at 30°C and pH 7.0 until the thiosulfate was depleted. Aeration was provided at a rate sufficient to maintain 60-100 μM O_2 in the culture medium. Air was supplemented with 5 mol% CO_2 to ensure a continuous availability of a carbon source. When the thiosulfate was depleted the optical density (at 460 nM) of the culture was approximately 1.0 and the T. denitrificans viable count was approximately 10^9 cells/ml. Following thiosulfate depletion the culture received two gas feeds, 0.9-1.1 mol% H_2S, 5 mol% CO_2 and balance nitrogen and air supplemented with 5 mol% CO_2. The aeration rate and agitation rate (300-400 rpm) were adjusted to produce steady-state oxygen concentration of 60-150 μM in the culture medium. The aeration rate was typically in the range of 200-400 ml/min. Foaming was controlled with the addition of 0.5 ml of a 1/10 dilution of General Electric AF93 silicone antifoam emulsion (previously autoclaved at 121°C, 205 kPa) approximately every 18-24 h. All other details of the operation of aerobic batch reactors were identical to those characteristics of the operation of anaerobic, batch reactors described earlier.

With the exception of providing aeration, the start-up and operation of continuous-flow aerobic reactors was virtually identical to the anaerobic systems. The nutrient feed solution was identical to the thiosulfate maintenance medium described in Table 1 with the exceptions that there was no thiosulfate or nitrate in the feed.

Oxidation of H_2S by Immobilized T. denitrificans

T. denitrificans was immobilized by co-culture with floc-forming heterotrophs obtained from activated sludge from the aerobic reactor of a refinery wastewater treatment system. T. denitrificans cells grown aerobically on thiosulfate and washed sludge were resuspended together in fresh thiosulfate maintenance medium without nitrate. The culture was maintained in a fed batch mode at pH 7.0 and 30°C with a gas feed of 5% CO_2 in air. With respect to the growth of T. denitrificans this medium was thiosulfate-limiting. When thiosulfate was depleted, the agitation and aeration were terminated and the flocculated biomass allowed to settle under gravity. The supernatant liquid was then removed and discarded. In this way the culture was enriched for T. denitrificans cells which had become physically associated with the floc. The volume was then made up with fresh medium and aeration and agitation restarted. This fed-batch cycle was repeated 5-6 times.

At the end of the fed batch cycles the biomass was allowed to gravity settle and was washed four times with 20 mM phosphate buffer (pH 7.0) to remove free-cell biomass. Floc were then resuspended in fresh medium (without thiosulfate) in the fermenter and sparged with 500 ml/min of 5% CO_2 in air and 50 ml/min of 1.0% H_2S in nitrogen.

Analytical

All gas analyses were conducted with a Perkin Elmer Sigma I Gas Chromatograph or Hewlett-Packard 5995 GC/MS. The Perkin Elmer featured a thermal conductivity detector with a detection limit for H_2S of 2-4 μM with a 0.25 ml sample at 101.3 kPa and 25°C. The column used was a 10-ft by 1/8-in. ID Teflon column containing 80/100 mesh Porapak QS (Waters Associates). The detection limit of the HP GC/MS for H_2S was found to be approximately 0.05 μM with a 0.25 ml sample at 101.3 kPa and 25°C. The column used was a 6-ft by 1/8-in. ID glass column containing 60/80 mesh Tenax GC (Alltech Associates).

Whole cell protein was determined by sonication followed by protein determination by the micro-modification of the Folin-Ciocalteau method (7,8). The protein content of T. denitrificans cells was determined to be 60% ± 3% by dry weight. Using this figure, protein analyses were converted to dry weight T. denitrificans biomass.

Nitrate was determined by the cadmium reduction method and nitrite by the diazotization method (9). Ammonium ion was determined by the Nessler method without distillation (9). Thiosulfate was determined by titration with standard I_2 solution with a starch indicator (10). Sulfate was determined turbidiometrically (9).

Total sulfide (H_2S, HS^- and S^{-2}) was determined by ion specific electrode using an Orion Research Model 94-16 sulfide/silver electrode and an Orion Research Model 701A pH/mV meter. Elemental sulfur collected by filtration on 0.45-μ Millipore Type HA filters was determined by reaction with cyanide to produce thiocyanate which was quantitated as $Fe(SCN)_6^{-3}$ (11).

Viable counts of T. denitrificans were determined by plating serial dilutions of culture medium on thiosulfate agar plates and incubating anaerobically at 30°C. Heterotrophs were quantitated by plating samples of culture medium on nutrient agar (Difco Labs) plates incubated aerobically at 30°C.

Free-cell or planktonic biomass in reactors containing immobilized T. denitrificans was estimated in terms of the optical density (460 nm) of the supernatant after medium samples were allowed to settle under gravity for 10 min. Settling properties of flocculated biomass were determined during the course of H_2S fermentation by periodically turning off all gas feeds and the agitation and measuring the height of the biomass in the fermenter at intervals for 20 min.

RESULTS AND DISCUSSION

Batch Growth of Planktonic T. denitrificans on H_2S

When H_2S was introduced to anaerobic or aerobic cultures of planktonic or free-cell T. denitrificans previously grown on thiosulfate, the H_2S was immediately metabolized with no apparent

lag. At initial loadings of 4-5 mmoles/h-g biomass the reactor outlet gas generally contained less than 2.0 μM H_2S. When H_2S was detected in the outlet gas, an increase in the agitation rate reduced the H_2S concentration to levels undetectable by GC/MS. The residence time of a bubble of feed gas (average diameter approximately 0.25 cm) under these conditions was 1-2 s. Less than 1 μM of total sulfide (H_2S, HS^-, S^{-2}) was observed in the reactor medium during periods of up to 36 h of operation. No elemental sulfur was detected; however, sulfate accumulated in the reactor medium as H_2S was removed from the feed gas (Figure 2). Oxidation of H_2S to sulfate was accompanied by growth, as indicated by an increase in optical density and protein concentration and a decrease in the NH_4^+ concentration as shown in Figures 2 and 3. Consumption of OH^- equivalents indicated that the reaction was acid producing. Nitrate was consumed under anaerobic conditions as expected (Figure 3), but no nitrite was observed to accumulate.

Sample material balances for batch aerobic and anaerobic H_2S oxidation are given in Table 2. Table 3 presents this type of data as stoichiometric ratios all relative to H_2S oxidized. The stoichiometries of aerobic and anaerobic oxidation of H_2S(g) by T. denitrificans in batch reactors were similar with respect to sulfate production and ammonium and hydroxide utilization. However, the biomass yield under aerobic conditions was only about 37% of that observed under anaerobic conditions. These results suggest that oxygen may be a growth inhibiting substrate for T. denitrificans growing on H_2S(g). Similar results have been reported by Justin and Kelley (12) for aerobic growth of T. denitrificans on thiosulfate.

Growth of Planktonic T. denitrificans on H_2S in a CSTR

T. denitrificans was readily cultured both anaerobically and aerobically on a continuous basis with a H_2S feed. Less than 2 mg/l of elemental sulfur was deterected in the reactors at any time. Nitrite was detected in some anaerobic reactors during start-up (up to 0.25 mM in one instance); however, no nitrite could be detected in any anaerobic reactor at steady state. The steady state concentration of total sulfide in each reactor was less than or equal to 1 μM. This was true throughout each experiment even during start-up. Typical behavior in approach to steady state in an anaerobic CSTR is illustrated by Figures 4 and 5. Small amounts of N_2O (< 40 μM) resulting from the incomplete reduction of nitrate were detected in the outlet gases from anaerobic reactors. With sufficient agitation H_2S was undetectable by GC/MS in the outlet gases of both aerobic and anaerobic reactors.

The stoichiometry of anaerobic and aerobic H_2S oxidation in continuous cultures is also given in Table 3. Under anaerobic conditions the yield of biomass was seen to be greater at the higher dilution rate as expected since a greater fraction of energy yielding substrate will be consumed to support growth as opposed to cell maintenance at higher growth rates. The true growth yield and maintenance coefficient for anaerobic H_2S oxidation by T. denitrificans were calculated by the method given by Pirt (13) to be 21.1 g dry wt biomass/mole H_2S and 1.74 mmoles H_2S/h-g dry wt,

respectively. At D=0.029 h^{-1} the average yield was 9.3 g biomass/mole H_2S oxidized. This compares favorably with that reported by Timmer-ten-Hoor (14,15) (9.41 g biomass/mole S^{-2}) for anaerobic growth of T. denitrificans on Na_2S as limiting substrate at D=0.030 h^{-1}. Under aerobic conditions biomass yield was relatively unaffected by the specific growth rate in the range of 0.030 h^{-1} to 0.053 h^{-1}. As indicated in Table 3 biomass yield under aerobic conditions in continuous cultures was lower than that observed under anaerobic conditions at comparable dilution rates.

Reactor Upset and Recovery

In those experiments described above, the H_2S feed rate was always less than the maximum rate at which the biomass was capable of oxidizing the substrate. If the maximum capacity of the biomass for H_2S oxidation is exceeded, inhibitory levels of sulfide will accumulate in the medium. In order to examine the behavior of a T. denitrificans reactor in an upset condition, the H_2S feed rate to aerobic and anaerobic batch and continuous flow reactors like those described in the previous sections was increased in a stepwise manner until H_2S breakthrough was obtained. At the point at which breakthrough occurred, N_2O was also detected in the outlet gas from anaerobic reactors in concentrations approximately equal to that of the H_2S in the feed gas. Analysis of the reactor medium from both aerobic and anaerobic reactors also indicated an accumulation of sulfide and elemental sulfur in the reactor. Sulfur balances for reactors operated under upset conditions showed that all of the H_2S removed from the feed gas could be accounted for in terms of sulfate, elemental sulfur, and sulfide in the medium. It was observed that the upset condition was reversible if the cultures were not exposed to the accumulated sulfide for more than 2-3 h. Reduction in H_2S feed rate following an upset condition reduced H_2S and N_2O concentrations in the outlet gas to preupset levels. In addition, elemental sulfur, which accumulated during upset, was rapidly oxidized to sulfate.

It is of importance to know at which H_2S loadings the specific activity of the T. denitrificans biomass will be exceeded resulting in upset. The maximum loading of the biomass under anaerobic conditions was observed to be in the range of 5.4-7.6 mmoles/h-g biomass. Under aerobic conditions, the maximum loading was observed to be much higher, 15.1-20.9 mmoles H_2S/h-g biomass.

Effect of Heterotrophic Contamination

The autotrophic medium used in these experiments will not support the growth of heterotrophs since there is no organic carbon source. However, early on in this study it was observed that if aseptic conditions were not maintained, a heterotrophic contamination developed in a T. denitrificans culture. Evidently T. denitrificans releases organic material into the medium in the normal course of growth or through lysis of nonviable cells which supports the growth of heterotrophs. In order to investigate the effect of heterotrophic contamination on the performance of a T. denitrificans CSTR, one anaerobic reactor which became contaminated was allowed to operate for an extended period of time. The reactor

was originally contaminated by two unidentified gram-negative heterotrophs which had distinctly different colony morphology on nutrient agar. After 145 h of operation, the reactor was injected with suspensions of four facultatively anaerobic heterotrophs in phosphate buffer. These were Pseudomonas chlororaphus ATCC 9446, Pseudomonas stutzeri ATCC 11607, Pseudomonas fluorescens ATCC 33512, and an unidentified gram-negative lab isolate. After 315 h of operation, a sample of Desulfovibrio desulfuricans ATCC 13541 in phosphate buffer was also injected. The Desulfovibrio quickly washed out. However, the total heterotroph concentration increased to about 10^8 cells/ml and leveled off. Apparently growth of the contaminants became limited by the availability of suitable carbon sources. The viable count of T. denitrificans at steady state was 5.0×10^9 cells/ml. The steady state composition of the culture medium and outlet gas condition were indistinguishable from that of a pure culture of T. denitrificans operated under the same culture conditions. These observations led to the efforts to immobilize T. denitrificans by co-culture with floc-forming heterotrophs described below.

Oxidation of H_2S by Immobilized T. denitrificans

The activated sludge used as a source of floc-forming heterotrophs in the immobilization of T. denitrificans contained many morphological forms of bacteria as well as fungi and protozoa. However, at the end of the fed-batch cycles (18 days on the average) in co-culture with T. denitrificans in thiosulfate maintenance medium, microscopic examination revealed only short (0.5 μ by 1.0-1.5 μ), gram-negative rods. T. denitrificans and Zoogoea ramigera are both short gram-negative rods. Zoogloea ramigera is the most common floc-forming bacterium found in activated sludge systems.

When $H_2S(g)$ was introduced into batch aerobic reactors containing immobilized T. denitrificans, the H_2S was immediately metabolized. With gas-liquid contact time of approximately 1-2 sec the outlet gas of the reactor typically contained less than 0.1 μM H_2S. Sulfate was observed to accumulate in the medium as H_2S was removed from the feed gas. The oxidation of H_2S was accompanied by growth as indicated by an increase in the total protein concentration and a decrease in the NH_4^+ concentration. In a typical experiment the oxidation of 205.5 mmoles H_2S was accompanied by the production of 204.9 mmoles sulfate and 614.7 mg biomass protein and the utilization of 16.2 mmoles NH_4^+. This stoichiometry is comparable to that given in Table 3 for the aerobic oxidation of H_2S by planktonic or free-cell T. denitrificans biomass.

As noted above, each time fermenters were sampled the sample was allowed to settle under gravity for 10 min and the optical density of the supernatant measured. At the time H_2S feed was initiated the OD_{460} was typically 0.25-0.32. As H_2S was removed from the feed gas the OD after settling was observed to actually decline indicating that no free-cell biomass accumulated in the culture medium. It appears that the growth of the autotroph T. denitrificans was balanced with the growth of the floc-forming heterotrophs through a commensal relationship in which the growth

of the heterotrophs was limited by organic carbon derived from T. denitrificans. The result was an immobilization matrix which grew with the T. denitrificans.

Figure 6 gives typical settling curves for the immobilized T. denitrificans biomass during the course of growth on H_2S. As shown here the settling properties of the biomass were relatively constant during these experiments. When the fed-batch cycles are coupled with the H_2S experiments, the settling properties of the biomass are seen to have been maintained for in excess of 25 days without external addition of organic carbon.

CONCLUSION

It has been demonstrated that the H_2S content of a gas can be reduced to very low levels by contact with an aerobic or anaerobic culture of planktonic or free-cell Thiobacillus denitrificans if the reactor is operated under sulfide-limiting conditions. Hydrogen sulfide was observed to be an inhibitory substrate; however, upset conditions produced by excess H_2S feed were readily detected and reversed. Hydrogen sulfide was oxidized completely to sulfate. Under aerobic conditions the maximum loading of the biomass was 2-3 times higher than that observed for anaerobic conditions.

T. denitrificans has also been immobilized in macroscopic floc by co-culture with floc-forming heterotrophs from an activated sludge treatment facility. Floc with excellent settling characteristics were produced which were subsequently used to remove H_2S from a gas stream bubbled through a batch aerobic culture. No organic carbon addition was required during enrichment for immobilized cells of T. denitrificans or during H_2S oxidation. No free-cell biomass was released into the medium during growth on H_2S. The stoichiometry and kinetics of H_2S oxidation by immobilized T. denitrificans were comparable to that observed in free-cell cultures. Immobilization of T. denitrificans will facilitate concentration of cell suspensions by gravity settling and cell recycle in a continuous system. A corresponding increaase in volumetric productivity is anticipated.

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TABLE 1. Growth Medium for Thiobacillus denitrificans

Component	per liter
Na_2HPO_4	1.2 g
KH_2PO_4	1.8 g
$MgSO_4 \cdot 7H_2O$	0.4 g
NH_4Cl	0.5 g
$CaCl_2$	0.03 g
$MnSO_4$	0.02 g
$FeCl_3$	0.02 g
$NaHCO_3$	1.0 g
KNO_3 (Anaerobic)	5.0 g
$Na_2S_2O_3$ (Grow up only)	10.0 g
Trace metal solution	15.0 ml
Mineral water	50.0 ml

TABLE 2. Material Balances: Aerobic and Anaerobic Oxidation of $H_2S(g)$ in Batch Reactors by T. denitrificans

	Anaerobic	Aerobic
H_2S oxidized	18.3 mmoles	86.0 mmoles
SO_4^{-2} produced	18.8 mmoles	81.8 mmoles
Biomass produced	246 mg	453 mg
NO_3^- consumed	27.0 mmoles	-
NH_4^+ consumed	2.2 mmoles	8.4 mmoles
OH^- consumed	31.8 meq	151.3 meq

TABLE 3. Stoichiometry of H_2S Oxidation by *Thiobacillus denitrificans*[a]

Reactor Type	Electron Acceptor	NO_3^-/H_2S (mole/mole)	O_2/H_2S (mole/mole)	SO_4^{-2}/H_2S (mole/mole)
Batch	NO_3^-	1.36		1.04
CSTR D = 0.029 h^{-1}	NO_3^-	1.30		1.03
CSTR D = 0.058 h^{-1}	NO_3^-	1.19		1.00
Batch	O_2		1.81	0.99
CSTR D = 0.030 h^{-1}	O_2			1.06
CSTR D = 0.053 h^{-1}	O_2			1.04

TABLE (continued)

Reactor Type	Electron Acceptor	NH_4^+/H_2S (mole/mole)	OH^-/H_2S (eq/mole)	Biomass/H_2S (g/mole)
Batch	NO_3^-	0.12	1.60	12.1
CSTR D = 0.029 h^{-1}	NO_3^-	0.09	1.37	9.3
CSTR D = 0.058 h^{-1}	NO_3^-	0.10	1.24	12.9
Batch	O_2	0.10	1.75	4.5
CSTR D = 0.030 h^{-1}	O_2	0.11[b]	2.38[b]	8.1
CSTR D = 0.053 h^{-1}	O_2	0.12	1.77	7.9

[a] Average of three or more determinations unless otherwise noted.
[b] Average of two determinations.

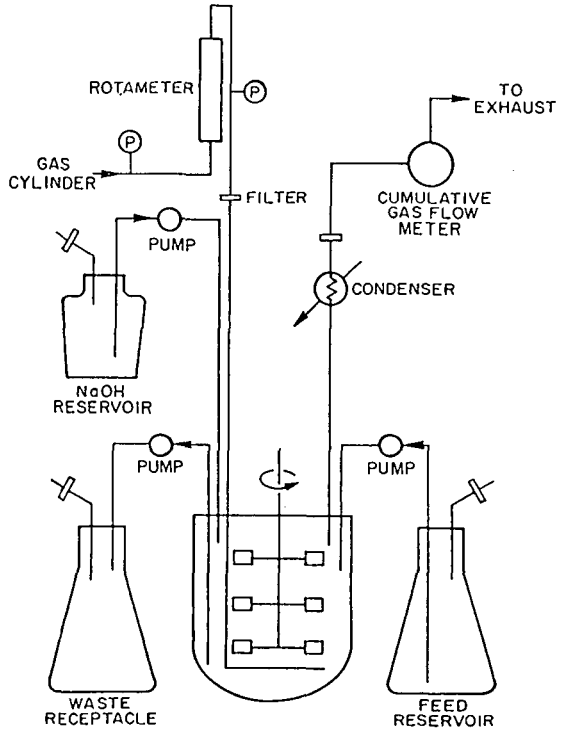

FIGURE 1. Schematic Diagram of Continuous Flow Fermentation System.

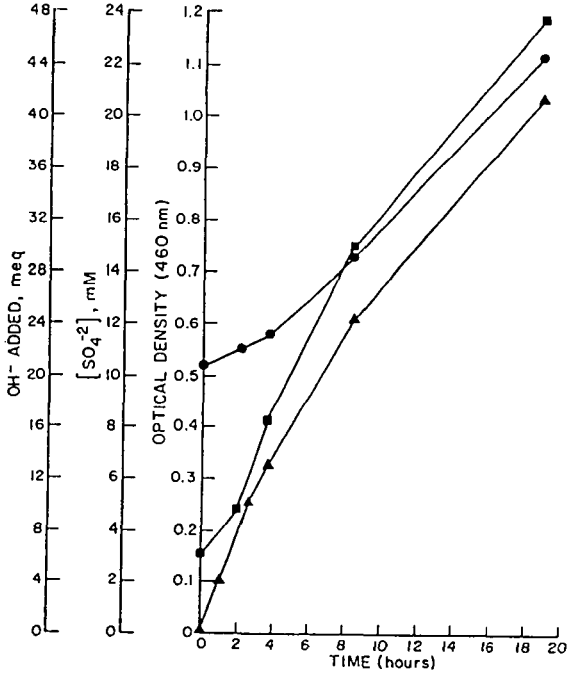

FIGURE 2. Optical Density, Concentration of Sulfate (SO_4^{-2}) and Hydroxide Ion (OH^-) Utilized in an Anaerobic T. denitrificans Batch Reactor Receiving 1.25 mmole/hr Hydrogen Sulfide (H_2S) Feed. OD (●); SO_4^{-2} (■); OH^- (▲).

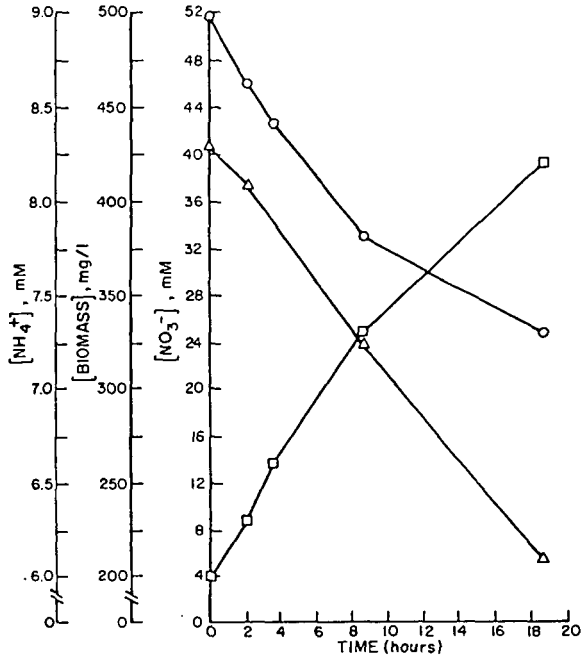

FIGURE 3. Concentrations of Nitrate (NO_3^-), Biomass and Ammonium (NH_4^+) in an Anaerobic T. denitrificans Batch Reactor Receiving 1.25 mmoles/hr Hydrogen Sulfide (H_2S) Feed. NO_3^- (O); NH_4^+ (Δ); Biomass (□).

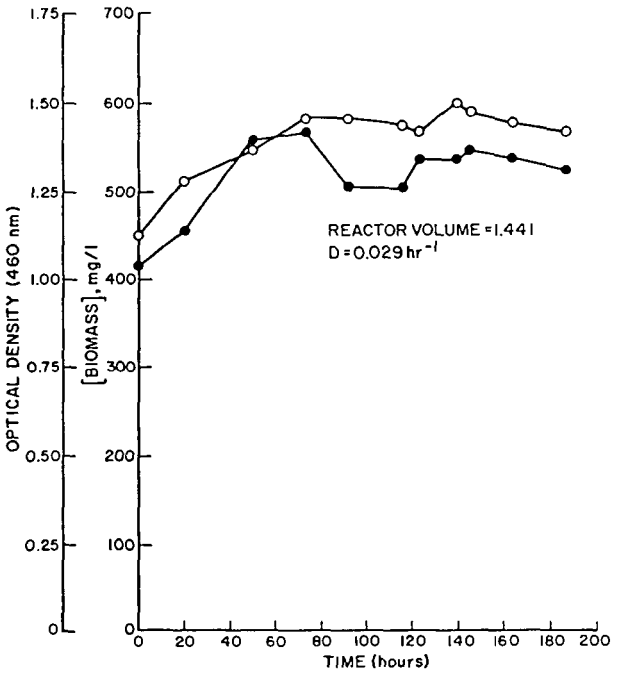

FIGURE 4. Concentration of Biomass and Optical Density in an Anaerobic T. denitrificans CSTR Receiving 2.6 mmoles/hr Hydrogen Sulfide (H_2S) Feed. OD (O); Biomass (●).

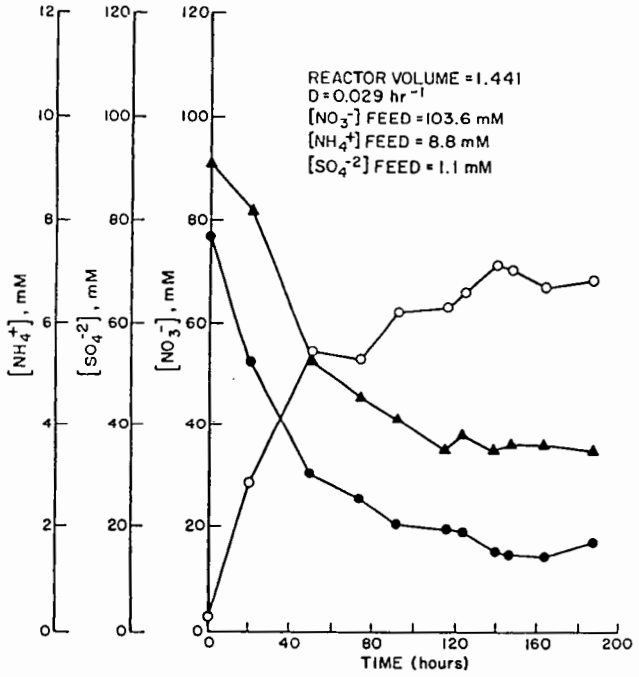

FIGURE 5. Concentrations of Nitrate (NO_3^-), Sulfate (SO_4^{-2}) and Ammonium (NH_4^+) in an Anaerobic T. denitrificans CSTR Receiving 2.6 mmoles/hr Hydrogen Sulfide (H_2S) Feed. NO_3^- (●); SO_4^{-2} (○); NH_4^+ (▲).

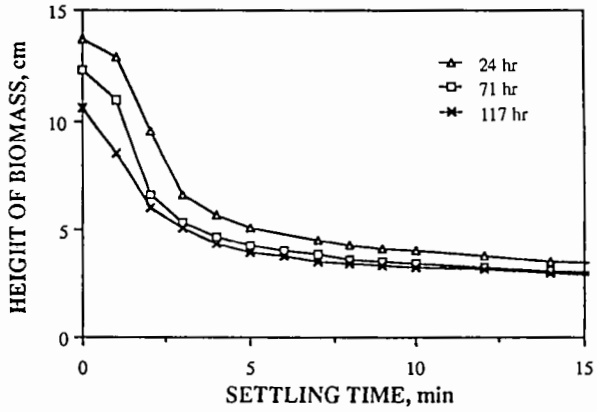

FIGURE 6. Height of Biomass in Settling Test in Marubishi MD 300 Fermenter.